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TITLE: Alleviating Autonomic Dysreflexia after Spinal Cord Injury

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14. ABSTRACT

Autonomic dysreflexia (AD) is a life-threatening dysfunction in which some stimulus below the level of SCI triggers extreme hypertension accompanied by bradycardia. It is thought to develop from 1) aberrant plasticity and 2) the loss of tonic input onto sympathetic preganglionic neurons (SPN) in the spinal cord that drive cardiovascular control. Both of these result in increased, unchecked activity of the SPN, leading to hypertension. This study aims to restore and/or form circuitry to normalize SPN activity. One potential means to achieve this is to promote the regeneration of appropriate axons to restore more normal SPN innervation. We have previously shown that we are able to promote robust functional axonal regeneration using a combination of transplantation and inhibitory matrix modulation (with chondroitinase). We have preliminary data indicating that modulation of microtubule dynamics (with monastrol) enhances this regeneration. We also have pilot data indicating the restoring innervation to the SPN diminishes AD. Another means to normalize SPN activity is pharmacologically. Pre-treatment with gabapentin (GP) has been shown to mitigate induced AD. However, whether post-treatment with GP effectively diminishes AD has not yet been demonstrated.

15. SUBJECT TERMS

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INTRODUCTION

Spinal cord injury (SCI) is a devastating event sustained by our servicemembers and as many as 1.3 million Americans that results in the disruption of normal autonomic function, including cardiovascular control. Up to 70%-90% of patients who have sustained a high SCI (above thoracic level 6 of spinal cord) suffer from the serious and life-threatening complication of autonomic dysreflexia. Autonomic dysreflexia manifests as sudden and extreme hypertension that is usually triggered by an annoying noxious or non-noxious stimulus below the level of injury, frequently initiated in the bladder or bowel. It is a key contributor to cardiovascular disease, the leading cause of morbidity and mortality for chronically injured individuals with SCI. However, the only current treatments available to treat autononic dysreflexia are palliative and involve pharmacological vasodilators that merely help to manage the symptoms of autonomic dysreflexia after an episode has already been initiated. Thus, developing treatments to help prevent the development of this syndrome or decrease its severity is highly important.

Significant progress has been made in identifying the mechanisms behind the development of autonomic dysreflexia after SCI. Firstly, the interruption of tonic, inhibitory control from supraspinal and intraspinal pathways that normally regulate the function of sympathetic preganglionic neurons located within the spinal cord which, in turn, help control cardiovascular function, plays a major role. The severity of autonomic dysreflexia symptoms is inversely correlated with the amount of spared spinal cord tissue, suggesting that sparing (or restoring) even a small amount of this normal input may result in a significantly improved quality of life. Another cause of autonomic dysreflexia is aberrant plasticity of spinal circuits that increase activity of the sympathetic preganglionic neurons. **We will test the hypothesis that AD can be mitigated by targeting these two very different root causes – i.e. promoting axonal regeneration to restore more normal innervation of sympathetic preganglionic neurons and pharmacologically inhibiting hyperexcitability.**

KEYWORDS

Autonomic dysreflexia, spinal cord injury, transplantation, axon regeneration

ACCOMPLISHMENTS

Through 9-30-2014 through 9-30-2015, we focused our efforts on the first 3 subtasks within the first Specific Aim (to test the hypothesis that restoring innervation of SPN in a subchronic, high-thoracic spinal cord transection model will mitigate autonomic dysreflexia).

The first subtask was to obtain IACUC and ACURO approvals. This was accomplished.

The second subtask was to determine the neuronal origin of axons that emerge from peripheral nerves grafted into a thoracic level 3 (T3) transection site. To do so, animals were transected at T3, segments of pre-degenerated peripheral nerve was grafted into the lesion cavity, and the distal graft-host interface was treated with either vehicle, monastrol (kinesin-5 inhibitor), chondroitinase (to digest inhibitory matrix molecules within the glial scar), or monastrol combined with chondroitinase. One month later, the retrograde tracer FluoroGold was injected into spinal cord caudal to the graft to identify neurons that had regenerated axons through the graft and back into host spinal cord. Animals were perfused a week later. Spinal cord and brain tissue was harvested, sectioned, and mounted onto slides for microscopic analysis. Representative images of the tissue can be seen below in Figure 1. In both control animals treated with PBS and DMSO vehicle and animals treated with ChABC and monastrol, neurofilament (red, B, F) axons grew into the nerve grafts. No FluoroGold+ cells (blue, C) are located in astrocyte-rich spinal cord tissue (shown in green, A, E) rostral to the transection/peripheral nerve graft in the control animals, suggesting that no axons regenerated out of the grafts in the control condition where the scar is left intact, as expected. On the other hand, FluoroGold+ neurons are visible rostral to the transection (arrows, G), strongly indicating that the combination of ChABC and monastrol promotes regeneration across the graft interface and into

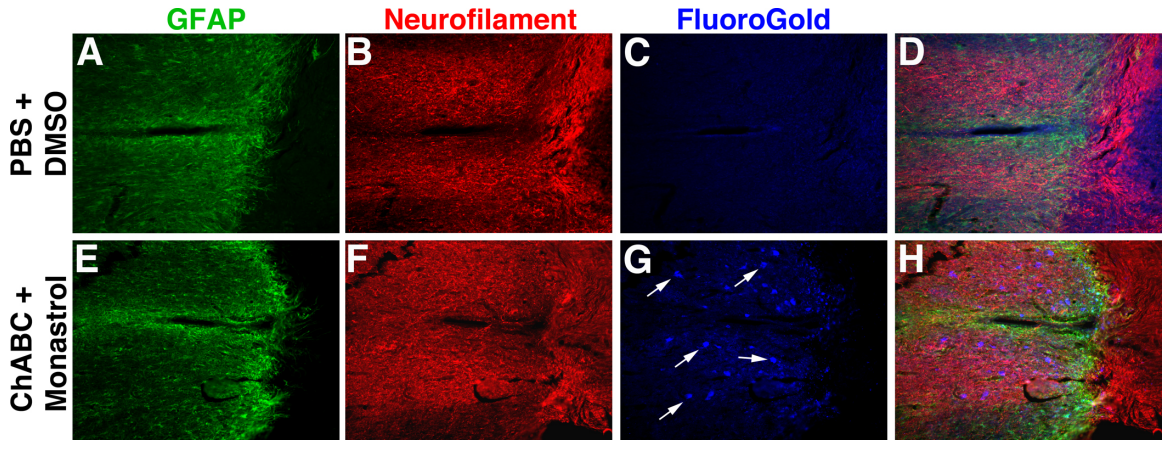

FIGURE 1. FluoroGold+ neurons rostral to a lesion in animals treated with ChABC and monastrol after application of the tracer FluoroGold caudal to the injury. This is indicative of enhanced regeneration of injured axons out of the graft with this combination.

distal spinal cord.

We counted the number of FG+ neurons in a subset of sections (Figure 2). We found that the most FluoroGold+ cells were found in the animals treated with chondroitinase+monastrol. Virtually all of these neurons were located within the spinal cord.

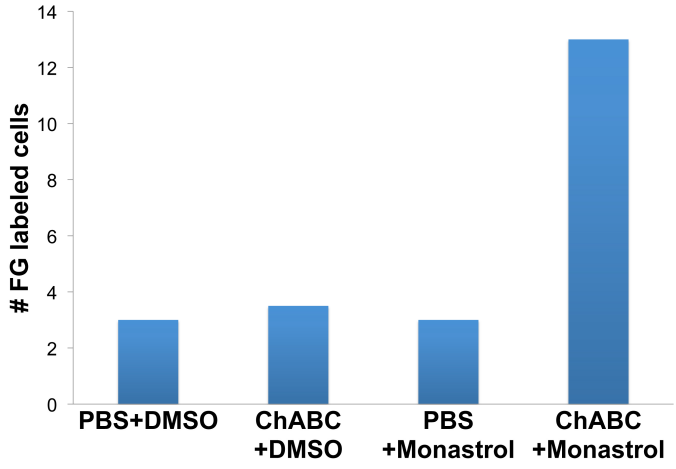

FIGURE 2. Quantification of retrogradely-labeled FluoroGold+ neurons. The most FluoroGold+ neurons were found in tissue rostral to the spinal transection site in the animals treated with chondroitinase + monastrol. This indicates that the most axon regeneration as promoted beyond a peripheral nerve graft with this treatment combination.

We also began work on the third subtask (to determine if combining a peripheral nerve graft, chondroitinase, and monastrol modulates colorectal distension-induced autonomic dysreflexia). Animals with thoracic level 3 transections receive peripheral nerve grafts and were treated with either PBS+DMSO (vehicle controls), chondroitinase+DMSO, PBS+monastrol, chondroitinase+monastrol intrathecally. We allow these animals to survive several months before implanting radiotelemeters into the femoral artery to allow us to measure blood pressure and heart rate in conscious animals. At least one week later, we assay blood pressure and heart rate in these animals at rest and after administering colorectal distension (CRD), a well-established experimental means to trigger an episode of autonomic dysreflexia. We are currently in the process of completing this subtask, including analyzing the data.

IMPACT

We have found that we can promote axon regeneration of propriospinal neurons out of a peripheral nerve that has been grafted into a high-thoracic spinal transection site. This was done most effectively when we treated the injury site with chondroitinase and monastrol. We are currently in the process of determining whether this regeneration mitigates autonomic dysreflexia after spinal cord injury.

CHANGES/PROBLEMS

Nothing to report

PRODUCTS

Nothing to report

PARTICIPANTS

Name:	*Veronica J. Tom, PhD*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	ORCID ID 0000-0002-3369-7575
Nearest person month worked:	*3*
Contribution to Project:	*Dr. Tom oversaw the progress of the experiments and helped to analyze the data*
Funding Support:	*N/A*

Name:	*Andrei Krassioukov, MD, PhD*
Project Role:	*Collaborator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Krassioukov consulted with respect to the experimental technique*
Funding Support:	*N/A*

Name:	*Shaoping Hou, PhD*
Project Role:	*Research associate*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*6*
Contribution to Project:	*Dr. Hou conducted the animal surgeries, performed histology on the tissue, and analyze the data*
Funding Support:	*N/A*

Name:	*Michelle Klaw*
Project Role:	*Research technician*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*2*
Contribution to Project:	*Ms. Klaw assisted with the post-operative animal care and helped with the histology*
Funding Support:	*N/A*

SPECIAL REPORTING REQUIREMENTS

An updated Quad chart has been included in the appendix.

Alleviating Autonomic Dysreflexia after Spinal Cord Injury

SC120077
W81XWH-14-1-0605

PI: Veronica J. Tom, Ph.D. **Org:** Drexel University **Award Amount:** $613,520

Study/Product Aim(s)

- To test the hypothesis that restoring innervation of SPN in a subchronic, high-thoracic spinal cord transection model will mitigate autonomic dysreflexia
- To test the hypothesis that combining restoration of SPN modulatory circuitry and pharmacological mitigation of hyperexcitability resulting from aberrant plasticity will result in greater mitigation of autonomic dysreflexia than either strategy alone

Approach

Autonomic dysreflexia (AD) is thought to result from two different root causes: 1) loss of tonic, inhibitory input from supraspinal and propriospinal neurons onto SPN; 2) hyperexcitability due to aberrant sprouting. We hypothesize that SPN activity after a high thoracic injury can be normalized by targeting each of these mechanisms – i.e. promoting axonal regeneration to restore normal innervation of SPN and pharmacologically inhibiting excitability – resulting in diminished AD.

Accomplishment: Surgeries to ascertain if grafting of peripheral nerve into a T3 transection site diminishes autonomic dysreflexia were continued. We began implantation of radiotelelmeters into the descending aorta via the femoral artery. We are also currently analyzing the data.

Timeline and Cost

Activities / CY	14	15	16	17
Determine if grafting diminishes AD				
Determine if gabapentin post-CRD is effective in diminishing subsequent episodes				
Determine if combining PNG and gabapentin is additive				
Estimated Budget ($K)	**$51k**	**$204k**	**$204k**	**$153k**

Updated: N/A

Goals/Milestones (Example)

CY14 Goal – Determine if grafting PN into a T3Tx site diminishes AD

☑ Determine neuronal origin of axons that emerge from the PNG

CY15 Goals – Determine if grafting PN into a T3Tx site diminishes AD

☐ Determine if combining a PNG, ChABC, and monastrol modulates colorectal distension-induced AD

CY16 Goal – Determine if gabapentin post-CRD diminishes severity of subsequent AD episodes

☐ Determine latest effective time point of gabapentin administration to alleviate AD

CY17 Goal – Determine if combining PNG-mediated regeneration and gabapentin has additive effects on mitigating AD

☐ Determine if combining the multiple strategies is additive

Comments/Challenges/Issues/Concerns

- None

Budget Expenditure to Date

Projected Expenditure: $204.5k
Actual Expenditure: $164.1k